

## HYDROGEN CYANIDE FUMIGATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 249

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(WITH TWO FIGURES)

The object of this work was to secure data on the manner in which green plants are affected by exposure to hydrocyanic acid; with particular emphasis on the resistance of the plants to this gas and the modification of this resistance by various factors, external and internal. A number of articles have been published concerning the effect of cyanide on animals. More recently its action as an enzyme paralyzer has been brought forward prominently. Information as to the action of cyanide on plants is of scientific interest, and certainly of practical value, for hydrocyanic acid finds important use as an insecticide in orchard and greenhouse practice. Circumstances have forced the discontinuance of this work, with many phases of it incomplete, but enough facts have been established, and enough new lines suggested, to warrant its publication in this incomplete form.

### Historical

Literature bearing on this problem is not abundant. The Department of Agriculture and several of the experiment stations, notably the California station, have published a number of bulletins dealing with fumigation as a commercial process; but the work done is of a kind which assists little in answering the fundamental physiological questions involved. The action of cyanide on the animal and in connection with various chemical processes has been thoroughly investigated, and from a consideration of these data we can gain much.

SCHÖNBEIN (8) first called attention to the inhibitory effects of hydrocyanic acid. He worked with the leaves of plants, and also with animal blood, and found that the presence of the acid prevented each of these materials from decomposing hydrogen peroxide. He concluded that the extremely poisonous action of cyanide on the

animal was due to an incapacitation of the red blood corpuscles, which caused suffocation, a conclusion still generally accepted. GEPPERT (3), and still later VERNON (10), who worked with animals, found this same inhibitory effect of hydrocyanic acid on respiration. They showed that if a lethal dose has not been given the organism recovers completely; that is, if the organism is not killed it is not injured in any way. SCHROEDER (9), using the fungus *Aspergillus niger*, made a long series of determinations on the effect of potassium cyanide on respiration. He confirmed the previous work and emphasized the fact that, if cells were not exposed too long, recovery was complete. He also did some work with ether, and decided that the inhibition of respiration caused by treatment with this anesthetic was quite different in character from inhibition caused by treatment with cyanide. He characterized diminution of respiratory rate through treatment with ether as a secondary effect, brought about by previous death of the tissue; while diminution of respiratory rate through treatment with cyanide is a primary effect, the cyanide directly inhibiting respiration and killing the tissue.

MATHEWS (5) has offered strong arguments favoring a contrary view, namely, that hydrocyanic acid and anesthetics both act primarily on the respiratory processes, each affecting these in exactly the same manner.

Another activity of cyanide, which has come into prominence in recent years, is its rôle as an "enzyme paralyzer" and more specifically its ability to check certain chemical reactions carried on in the laboratory. BREDIG and IKEDA (1), also LOEVENHART and KASTLE (4), have found that in the catalytic decomposition of hydrogen peroxide the addition of a very small amount of potassium cyanide completely stopped the reaction. They concluded that the cyanide was acting on the catalytic agent. MATHEWS and WALKER (6), who worked on the spontaneous oxidation of a very reactive amino acid, cystein, found that a very small amount of potassium cyanide checked this action. Their statement of the probable explanation of this is interesting, since the same reasoning is equally applicable to other inhibitions of oxidation processes by cyanide. "The proportion of cystein molecules in a condition to be oxidized

at any given time is extremely small; while the proportion of active potassium cyanide molecules is large. The number of active oxygen atoms is also small. If we further assume that the cyanide unites with the cystein at the same point where the oxygen ordinarily combined, then the results obtained are easily understood." In plant respiration, with cyanide present, we have the same general condition; that is, the oxygen of the air is not able to oxidize the plant compounds, and we may suppose that the cyanide has acted in the same way. In the case of respiration, of course, we may have a chain of several oxidations and reductions, and the exact point at which the cyanide intervenes is not known.

### Material

With the data at hand, while there is much on which to base inferences, there is nothing which tells of the behavior of an autotroph and the factors influencing this. Consequently, since this was pioneer work, it seemed highly desirable that it be done with normal green plants under normal conditions. The tomato was selected because it is easy to grow, sensitive to cyanide, and a plant commonly requiring fumigation in the greenhouse. Work under conditions such as those indicated is made difficult by the presence of factors not under control, which vary conditions with the different periods of time. In consequence of this it seemed best, in the case of most of the material experimented upon, not to try to carry on the work to its final expression, but merely to open up the way. The writer hopes at a later date to complete the work upon some of the more important phases with conditions carefully controlled.

### Responses

The response of the plant to varying concentrations of hydrocyanic acid was observed as indicated by the subsequent growth of the plant. Two main points were considered in deciding the effect of a given concentration of gas: (1) the growth rate of the plants after fumigation; (2) the appearance and growth character of the plants after fumigation. The method was to select 7 or 8 groups of plants, similar in all respects, and to fumigate these on successive nights. Suitable checks were kept, and the growth of the treated

plants was compared with the growth of the check plants during the *same* days. Many series, made up as indicated, were run, the experiments covering a period of 5 months. It is obvious that the slow growing plants of January are not wholly comparable with the rapid growing plants of April. This matter cannot be controlled,

TABLE I  
AVERAGE INCREASE OR DECREASE IN GROWTH DURING 12 DAYS  
FOLLOWING FUMIGATION

KCN per cubic feet	Plants measured	Percentage 1st to 4th days	Percentage 5th to 8th days	Percentage 9th to 12th days
0.001 gm.....	6	-16	+13	- 8
0.002 gm.....	10	-19.5	- 1.5	-11.5
0.003 gm.....	20	-11.5	-10.2	+ 9.3
0.004 gm.....	19	- 5.5	+21	- 7
0.005 gm.....	25	- 4.2	+11.4	+11.6
0.006 gm.....	30	- 5.7	+16	+13
0.007 gm.....	20	+17.7	+ 7	+ 6.5
0.008 gm.....	16	+33	+15.3	-18

TABLE II  
PERCENTAGE OF 4 DAY PERIODS SHOWING DECREASED GROWTH RATE AND ALSO PERCENTAGE SHOWING INCREASED GROWTH RATE

KCN	Percentage showing rate decrease	Percentage showing rate increase
0.001 gm.....	66.6	33.3
0.002 gm.....	83.6	0
0.003 gm.....	63.5	36.4
0.004 gm.....	50.0	41.6
0.005 gm.....	33.3	66.6
0.006 gm.....	33.3	55.5
0.007 gm.....	16.6	75.0

and introduces an element of error. The time of exposure, length of exposure, temperature, and moisture conditions were similar in all cases. Using the growth of the check lots as 100 per cent, the loss or gain of the treated lots was computed, and from these values the averages shown in tables I and II were secured.

Consideration of these data shows that with concentrations of cyanide up to 0.004 gm. per cubic foot of air space, the effects were

somewhat detrimental to the growth of the plant. With 0.004, stimulation and retardation effects seem to be about balanced; but at 0.005, and continuing up through 0.006 and 0.007, stimulation effects plainly dominate. The plants giving these responses retained after the treatment all appearances of normal growth and metabolism.

With the concentration of 0.008 gm. KCN the first injury appeared, although there was some variation in this regard. Thus in one series no injury appeared before 0.01 gm. KCN. The harmful effect of this injury was very marked. Growth records were taken for concentrations up to 0.016, but unfortunately, with these damaging strengths (0.008–0.016), growth records do not always indicate the true condition of the plants. False stimulation

TABLE III  
PERCENTAGE INCREASE OR DECREASE IN GROWTH FOLLOWING FUMIGATION

KCN per cubic foot	Percentage 1st to 4th day	Percentage 5th to 8th day	Percentage 9th to 12th day	Percentage 13th to 16th day
0.007 gm.: no injury.....	+14	+32	-33	-16
0.008 gm.: injury to parts of plants.....	+36	+44	-58	-44
0.009 gm.: injury to all plants..	-37	+ 2	-63	-58
0.01 gm.....	-27	-29	-66	
0.012 gm.....	-16	-40	-12	

was frequently shown. This is an increase in growth rate of plants obviously in poor condition. The explanation of this false stimulation is that the growing tips of plants are rarely killed, and in consequence of the loss of considerable leaf area through injury these growing tips are forced into renewed activity. Thus badly injured plants in the course of a month's time form entire new tops, although old injured leaves drop off, for the most part. Usually, however, when injury occurred the depressing effects were so strong that this increased growth rate was very transient in character, or did not show at all, as is seen in table III.

A characteristic feature of this injury is the duration of the depressive effects. The plants were still far below normal at the

end of the 12 or 16 day period recorded. The following results were secured:

GRAM KCN PER CUBIC FOOT OF AIR SPACE

- 0.001
- 0.002, temporary depressive effects
- 0.003
- 0.004, intermediate effects
- 0.005
- 0.006, temporary stimulative effects
- 0.007
- 0.008, relatively permanent depressive effects
- 0.009
- 0.01

It is interesting to note that the greenhouse white fly (*Aleyroides*) was killed at a concentration of 0.006 gm. KCN. Another point worthy of emphasis is that the actual difference in amount between a concentration of cyanide which brings about noticeable injury to the plants, and one which does no harm, may be very small indeed. This compares very well with previous statements (3, 9) that cyanide either killed or that recovery was complete.

There is not much work which can be cited in substantiation of the mixed stimulative and depressive effects found. In the work with hydrogen peroxide (4) very low concentrations of cyanide accelerated the reaction. Again it was discovered (7) that cyanide hastened the oxidation of an amino acid when the amino acid was present in an impure state. With these two exceptions the cyanide literature bearing on this work deals with inhibitory actions.

## Factors

### EXTERNAL FACTORS

MOISTURE.—In considering moisture as an external factor we deal only with the effect of free water on the leaf surface of the plants fumigated. It is common knowledge among greenhouse men that if plants go into the fumigation wet, considerable injury is often induced. In testing this I have found that some species are made more susceptible to injury by wetting the leaves, while other species are not visibly affected. The tomato is in this latter class. On closer observation it was found, in the case of the tomato, that

not only did the free water have no detrimental action, but actually, under certain conditions, gave beneficial results. This was true in the cases where the strength of fumigation was not sufficient to injure the plants (below 0.008 gm. KCN):

## GROWTH DURING 12 DAYS FOLLOWING FUMIGATION

	Dry plants	Wet plants
Lot 1.....	100 per cent.....	127 per cent
Lot 2.....	100 per cent.....	115 per cent
Lot 3.....	100 per cent.....	139 per cent

With the following data, however, the fumigation was strong enough to injure the plants:

	Dry plants	Wet plants
Lot 1.....	100 per cent.....	115 per cent
Lot 2.....	100 per cent.....	99 per cent
Lot 3.....	100 per cent.....	100 per cent

Thus wetting the leaves had a beneficial effect if the fumigation was not strong enough to cause injury. With the appearance of injury this beneficial action ordinarily disappeared. The results just given are from experiments performed at wide intervals, and the plants used varied greatly as to size and age. Moisture present in pans as free water surfaces or saturated soil gave negative results.

TEMPERATURE.—The effect of temperature on plant resistance was determined qualitatively only. Excessive temperatures during the period of fumigation very materially increase the amount of injury. A variation of 20° F. has a marked effect; thus plants fumigated at 75° F. suffered much less injury than others which were fumigated at 95°. In what way temperature affects the plant, to cause these changes, has not been determined. One possible explanation is that the effect is through changes in permeability of protoplasm, such as RYSELBERGHE, LEPESCHKIN, and ECKERSON have shown to occur, or even in permeability of wall structures. That it may be due to a change in the rate of chemical reaction is of course possible.

LIGHT.—Light undoubtedly exerts a direct action on plant resistance, but the conditions did not permit accurate observation on this point. Here permeability changes as well as catalytic effects of light appear possible. The indirect action of light as a regulator

of stomatal aperture and in connection with photosynthetic activity will be discussed later.

#### PLANT FACTORS

PROTECTIVE MEMBRANES.—Most plants which are highly resistant to cyanide are characterized by having thickly cutinized or suberized epidermal membranes which serve as a protection. As further evidence of the protective power of these, *Tradescantia zebrina* is made perceptibly more resistant by coating the upper surfaces of the leaves with Blackman's wax. The *Tradescantia* leaf has no stomata on the upper surface, and the reduction in injury must be due to the wax covering, thus making the thin epidermis relatively impervious. This increased resistance, when unobscured by a large amount of stomatal activity, is very marked. It is by no means possible to explain all differences in resistance on the basis of protective membranes, however. The radish endures without injury 3 times the strength of fumigation which a tomato endures, yet microchemical examination reveals but little difference in the cuticular development.

STOMATA.—The stomata seem to be the most important single factor in determining the amount of injury resulting from hydrocyanic acid fumigation. To ascertain the extent of their influence experiments were conducted in the following manner. Fumigations were run at various times of day and night, using like strengths of cyanide. Each lot of plants exposed included tomatoes and *Tradescantia zebrina*, the leaves of the latter being painted in various ways with Blackman's wax. After the beginning of each fumigation samples of epidermis were taken from several species with large stomata (*Geranium* and *Tradescantia*), and the amount of stomatal opening determined under the microscope.

#### FUMIGATIONS CONDUCTED ON A VERY DARK RAINY DAY (0.02 GM. KCN PER CUBIC FOOT)

EXPOSURE 1:30 TO 3:30 P.M.

Tomatoes.....	Badly injured
<i>Tradescantia</i>	
Leaves under surface coated . . .	Uninjured (stomatal surface closed)
Leaves upper surface coated . . .	Badly injured (stomatal surface open)
Leaves untreated.....	Killed (stomatal surface open)
Average stomatal opening 1:30 P.M., 3.5 $\mu$	



## EXPOSURE 5:30 TO 7:30 P.M.

Tomatoes.....	Slightly injured
<i>Tradescantia</i>	
Leaves under surface coated..	Uninjured (stomatal surface closed)
Leaves upper surface coated..	Uninjured (stomatal surface open)
Leaves untreated.....	Slight injury (stomatal surface open)
Average stomatal opening 5:30 P.M., almost all stomata were closed.	

## EXPOSURE 11:30 P.M. TO 1:30 A.M.

Tomatoes.....	Occasional slight injury
<i>Tradescantia</i>	
Leaves under surface coated..	No injury (stomatal surface closed)
Leaves upper surface coated..	No injury (stomatal surface open)
Leaves untreated.....	No injury (stomatal surface open)
Average stomatal opening, none.	

It was evident that the closing of the stomata greatly increased the resistance to fumigation. Approximately speaking, if 100 per cent were to represent the injury at 1:30 P.M., by 5:30 P.M. it had dropped to 10 per cent, and by 11:30 to 2 per cent. Why the plants were most resistant at the very late period was not entirely apparent, the stomata at 5:30 P.M. being, with very few exceptions, as tightly closed as at 11:30 P.M. Miss ECKERSON, however, has found that stomata in certain portions of some leaves lag behind as to closing time. During sunny weather there are great variations of light and temperature in the course of a day and night. This is in contrast with conditions during dark weather. In a sunny period, however, considerable work was done between 6:30 P.M. and midnight.

It was found that stomatal activity, as indicated by injury to the hypostomatous *Tradescantia*, continued on a gradually diminishing scale till 8:45 P.M. (about 2 hours after sundown). The stomata at this time were found to be almost closed. A noticeable fact was that at the later periods the correspondence between the amount of stomatal opening and the injury was not absolute. The injury at 8:00 P.M. was not so great as would have been expected from the size of the openings. In sunny weather, as in dark, there was a gradual increase in resistance of the plants during the night. The maximum of resistance was reached during the period between 11:30 P.M. and 1:00 A.M.

In conclusion it may be said that the amount of injury follows the stomatal movement rather closely. A fumigation at 5:30 P.M. on a dark day was about equivalent in injury to one at 8:30 P.M. on a bright day.

#### CHEMICAL FACTORS

**WATER.**—It was early observed that there were rather wide differences in the resistance of tomato plants grown under various conditions. It was found that when plants grew rather slowly, with a high chlorophyll content per unit area, they were very resistant to the hydrocyanic acid. Plants growing rapidly, with a low chlorophyll content per unit area, were very susceptible to injury from the hydrocyanic acid. Thus, judging solely by intensity of color, it was possible to select from a large group of plants 2 lots differing widely in their ability to withstand injury. Variations in water supply seemed to be the underlying cause of these differences, although other conditions will produce similar characters. To test this the following experiment was conducted: Twenty-four plants in vigorous growing condition were selected and divided into 2 lots. Lot 1 was watered only enough to keep growing, while lot 2 was watered abundantly. After 10 days, plants from lot 1, now dark green, were fumigated and found very resistant. Plants from lot 2, which were light green, were very easily injured by fumigation. An exhaustive chemical analysis of these plants was not made, but preliminary tests revealed one significant fact: the resistant plants (lot 1) had a greatly increased carbohydrate content. The reducing sugars claimed attention as being the most reactive of these substances, and also the ones most concerned in plant respiration. Determinations of the reducing sugar content of the leaves of lots 1 and 2 gave the following results (the samples were taken at 5:15 P.M.): lot 1, non-resistant leaves, 0.108 per cent calculated as dextrose per unit weight of green tissue; lot 2, resistant leaves, 0.57 per cent dextrose per unit weight of green tissue. Thus the resistant plants had much more reducing sugar. The actual amount is not large in either case, but the relative difference is great, lot 2 being 5 times as rich in reducing sugars as lot 1. The dry weight of the plants of lot 2 averaged little more than 1.5 times the dry weight of lot 1.

A possible rôle of reducing sugars as determiners of resistance was further tested by arbitrarily changing the sugar content of plants and observing the effects on resistance.

(1) Plants were placed in a dark box for 48-72 hours. This treatment brings the reducing sugars in the leaves practically to zero at the end of these periods. These plants, when fumigated, were found to be very easily injured.

(2) Plants treated exactly the same were taken from the dark box 12 hours before the fumigation and infiltrated with a glucose solution. They were returned immediately after the infiltration to the dark box, the exposure to light not exceeding 20 minutes. Plants thus made rich in glucose were highly resistant to cyanide injury. The following are extracts from data collected on this point:

FUMIGATION WITH 0.012 GM. KCN PER CUBIC FOOT, INFILTRATED AT 9:30 A.M.  
AT 6 CM. MERCURY PRESSURE

DARK BOX PLANTS

Checks.....Bad injury

INFILTRATED PLANTS

0.25 per cent glucose.....Slight injury  
0.50 per cent glucose.....No injury  
1.00 per cent glucose.....No injury (from fumigation)

FUMIGATION WITH 0.14 GM. KCN, INFILTRATED AT 10:00 A.M. AT 5 CM.  
MERCURY PRESSURE

DARK BOX PLANTS

Checks.....Very great injury

INFILTRATED PLANTS

0.90 per cent glucose.....No injury (from fumigation)  
0.75 per cent glucose.....No injury  
0.60 per cent glucose.....Very slight injury

With concentrations of glucose 0.9 per cent or above there was some injury to the plants from plasmolysis; such injury was distinct from that due to cyanide and caused no confusion. These experiments were repeated many times with uniformly decisive results. Frequently the check plants (not infiltrated) had their entire tops killed, while the treated plants showed little or no injury.

With these data in hand it is possible to say that for plants to be "normally" resistant they must have a fair content of reducing

sugar. MEYER has made determinations of the amounts of carbohydrates during the course of the day and night. Working with the leaves of *Tropaeolum*, he found that reducing sugars were at a minimum during the day but started to increase with nightfall. Analyses of tomato leaves yielded similar results; when the weather was bright the maximum reducing sugar content was found at 1:00-2:00 A.M. During dark weather this gradation was found to be much disturbed, as would be expected. The following figures were secured from the tomato leaves during such a period:

REDUCING SUGARS; WEATHER VERY DARK AND RAINY

1:30 P.M.,	0.414	per cent, calculated as dextrose (net weight)
5:30 P.M.,	0.335	per cent, calculated as dextrose
11:30 P.M.,	0.285	per cent, calculated as dextrose

Presence of other factors made it impossible to ascertain what effect these variations had on resistance. There seems to be little doubt that the glucose in the plant acts as a protective agent against injury by cyanide. Considering glucose to have a direct effect, there are several possibilities concerning the manner of this action. (1) It may protect the plant by supplying an excess of molecules to unite with the cyanide entering. Cyanide does unite readily with glucose. (2) There is much evidence in physiological experimentation, with both plants and animals, showing that an excess of glucose present will temporarily take the place of missing oxygen. Asphyxiated animals produce glucose in excess amounts, other compounds being broken down. Plants, in absence of oxygen, behave normally for a time when glucose is supplied. It may very readily be that the protective action of the glucose is an indirect one, working through other channels. Thus it may possibly modify stomatal action.

What goes to make up a resistant plant, and under what conditions is it most resistant? We have given but little attention to the first of these questions; hence the broad problem of why certain species of plants are much more resistant to cyanide than others will be left without attempting an answer. Rather exhaustive comparative studies seem to be the only possible way of solving this.

Concerning the conditions under which plants are most resistant, the following statements are possible:

The resistance of the tomato to cyanide is increased by the presence of water on the leaf surface, but with some species wet leaves increase injury. Temperature should be moderately low. Light intensity should be low during the day preceding the fumigation. The plants should go into the fumigation with the stomata closed. A large amount of reducing sugars in the plant is correlated with maximum resistance. Checking growth by reducing water supply, during the period preceding the fumigation, increased resistance.

The action of cyanide on entering the plant is a very interesting question, and all evidence favors the conclusion that cyanide combines readily with the substances there. In certain species of plants cyanides in the form of glucosides occur, and on these naturally occurring cyanides a great deal of work has been done. The investigators (12, 13) are unanimous in considering that this cyanide is never in an uncombined state. Granting then that the hydrocyanic acid, on entering the plant, unites quickly with plant compounds, there may still be variations in the type of union. Thus possibly there may be adsorption under certain conditions and under other conditions chemical combination.

Methods for the determination of cyanide in plants have been devised (2, 11), but they are rather laborious. One method is based on the fact that a cyanide in a picric acid-sodium carbonate solution gives a red color. The depth of the color, as compared with a standard range, is the basis of estimation. The coloration is due to the strong reducing action of the cyanide, but unfortunately the cyanide is not the only substance present in the plant which gives the reaction. For accurate quantitative work it is necessary to isolate the cyanide compounds. This was not done at this time; instead, estimations were made on unit weights of leaf tissue. The leaves were put in the picric acid-sodium carbonate solution, and observations were made after 24 hours by means of a colorimeter. The phrase "reducing substance content" is used because, as already stated, the cyanides are not the only things reacting. The variation in values secured, during the course of a series of

fumigations, is very interesting. In the data T represents an empirical measure.

FUMIGATION STRENGTH IN GM. KCN PER CUBIC FOOT	REDUCING SUBSTANCE CONTENT
0.003.....	T 7.5
0.004.....	T 8
0.005.....	T 8
0.006.....	T 8.5
0.007.....	T 9
0.008 point of first injury.....	T 4
0.009.....	T 3.5
0.010.....	T 3.5
0.012.....	T 2.5
0.016.....	T 2
0.020.....	T 1.5

The sudden drop at the point of first injury was very noticeable; thus from T 9 to a value less than half. Considering this drop as an index of chemical changes, it agrees well with the results of the growth experiments recorded at the beginning of the paper. Every indication was found, also, that the action of the cyanide, at a concentration just below the point of external injury, was radically different from its action at and beyond this point.

There are two main types of injury resulting from cyanide fumigation: (1) the killing of definite areas of leaf and, much more rarely, stem tissue. This injury is always localized on the younger portions of the plant. (2) Injury in the form of an epinastic response. This is frequently found in cases where the fumigation was just a trifle too strong. As a rule, under these circumstances, there is no apparent injury to the plants for a period of 5-7 days after the fumigation, and then a twisting of certain leaves (epinasty) becomes apparent. This kind of injury is quite distinct from the distortion which arises through an excessive killing of tissue.

The drawings in fig. 1 were secured after fumigating, with a damaging strength, a house containing tomato plants of different ages. The movement of the injury from the inner portions of the leaf in the old plants to the outer portions of the leaf in young plants was noticeable. The drawings show all the injured leaves from representative plants. Even with the very large plants the injury did not extend below the third or fourth leaf from the tip.



FIG. 1.—Injury from cyanide fumigation to tomato plants ranging in age from seedlings to mature plants (*a, b, c, d*); the injury is shown by the black areas, and it will be noted that the leaf tissues killed in the small seedlings (*a*) are peripheral, and also more or less apical.

There are several possible explanations of this localization on the younger portions. The epidermal membranes are not so well developed in the young leaves and it is easier for the cyanide to enter. The younger and more rapidly growing portions of a plant are the first to suffer in absence of oxygen. It may be that the very reactive state of the protoplasm in these parts makes them less resistant.



FIG. 2.—Older plants, in which there is a steady change in the location of the injured areas, until with old bearing plants the killed area is basal, and is not limited to the margin of the leaf.

The rapidity with which cyanide can kill the plant tissue is another point of interest. The plant can live in the total absence of atmospheric oxygen for some time. However, 5 hours after the beginning of a damaging fumigation it was possible to see the dead leaf areas, dark and water-soaked in appearance.

It may be said that there are several things which stand out prominently as causes for the unsatisfactory results often secured from greenhouse fumigation with cyanide. First, there is a lack of



appreciation of the necessity for delicate regulation of amounts of cyanide. Every separate greenhouse varies in its ability to retain the gas, and it is not alone the amount of cyanide which is put in that counts, but rather the amount which is retained. Thus definite recommendations cannot be given more than it is possible to give a universal fertilizer formula. Second, it is the usual thing to start the fumigation during the latter part of the afternoon when the stomata are still open. This is sure to induce excessive injury. It is safe to start fumigation 2.5-3 hours after sundown on a bright day, or at sunset on a very dark day.

### Summary

1. Different concentrations of hydrocyanic acid gas gave effects ranging from stimulative to depressive. The maximum of beneficial results was secured from concentrations deadly to insect life, but just a little below the point of first injury to the plant.

2. External factors having important action on the resistance are as follows: (a) wetting the leaves had a beneficial effect on the tomato; (b) reduced temperature and low light intensity during the day preceding fumigation increased resistance.

3. Injury closely paralleled the stomatal movement, increasing as the size of stomatal aperture increased.

4. A higher or lower water supply in the soil affected resistance, through hastening or retarding the growth rate. Rapid growing plants were susceptible to injury, while slow growing plants were more resistant.

5. High reducing sugar content seemed to be correlated with maximum resistance.

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## LITERATURE CITED

1. BREDIG, G., and IKEDA, K. I., Anorganische Fermente II. Zeitschr. Physikal. Chem. **37**:8-16. 1901.
2. CHAPMAN, A. C., The colorimetric estimation of hydrogen cyanide. Analyst **35**:469-477. 1910.
3. GEPPERT, J., Über das Wesen der Blausäurevergiftung. Zeitschr. Klin. Medicin **15**:208-307. *pl. 1.* 1889.
4. LOEVENHART, A. S., and KASTLE, J. H., On the catalytic decomposition of hydrogen peroxide and the mechanism of induced oxidations. Amer. Chem. Jour. **29**:397-436. 1903.
5. MATHEWS, A. P., The action of ether on an anaerobic animal tissue. Jour. Pharmacol. and Exper. Therapeutics **2**:231-238. 1910.
6. MATHEWS, A. P., and WALKER, S., The action of cyanides and nitrites on the spontaneous oxidation of cystein. Jour. Biol. Chem. **6**:29-37. 1909.
7. ———, The spontaneous oxidation of cystein and the action of iron and cyanides upon it. Journ. Biol. Chem. **6**:289-298. 1909.
8. SCHÖNBEIN, C. F., Über das Verhalten der Blausäure zu dem Blutkörperchen und den übrigen organischen das Wasserstoffsperoxide katalysirenden Materien. Zeitschr. Biol. **3**:140-144. 1867.
9. SCHROEDER, H., Über den Einflusse des Cyankaliums auf die Atmung von *Aspergillus niger*. Jahrb. Wiss. Bot. **44**:409-481. 1907.
10. VERNON, H. V., The effect of hydrocyanic acid. Jour. Physiol. **35**:70-73. 1906.
11. VIEHOVER, A., and JOHNS, C. O., Determination of small amounts of hydrocyanic acid. Jour. Amer. Chem. Soc. **37**:601-607. 1915.
12. VIEHOVER, A., JOHNS, C. O., and ALSBERG, C. L., Cyanogenesis in plants. Jour. Biol. Chem. **25**:141-150. 1916.
13. WILLAMAN, J. J., The estimation of HCN and the probable form it occurs in in *Sorghum vulgare*. Jour. Biol. Chem. **29**:25-45. 1917.